IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

STADDON *et al.*

Appl. No.: (To be assigned; Continuation of U.S. Appl. No. 08/648,182)

Filed: (Herewith)

For: **Use of an Agent Which Modulates Tyrosine Phosphorylation for Modulating the Permeability of a Psychological Barrier**

Art Unit: (To Be Assigned)

Examiner: (To Be Assigned)

Atty. Docket: 0623.0410001/EKS/BJD

Preliminary Amendment and Submission of Sequence Listing

Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of prosecution in the above identified matter, Applicants submit the following amendments and remarks. This Preliminary Amendment is provided in the following format:

(A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;

(B) Starting on a separate page, appropriate remarks. *See* 37 C.F.R. § 1.121 and MPEP § 714; and

(C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and

any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Title:

Please delete the Title of the application and substitute therefor the following Title:

Modulating the Permeability of a Physiological Barrier With an Agent that Modulates Tyrosine Phosphorylation .

In the Specification:

Please amend the specification as follows:

Please delete page 1 of the specification, and substitute therefor page 1 that is appended hereto as a substitute page.

At page 5, please delete the paragraph appearing at lines 8-18, and substitute therefor the following paragraph:

BRIEF SUMMARY OF THE INVENTION

The present invention is based, in contrast, on the surprising discovery that tyrosine protein phosphorylation is crucial to the control of the permeability of tight junctions in both epithelial and endothelial cells; tyrosine protein phosphorylation may therefore be manipulated to control

the permeability of the blood-brain and other physiological barriers. Decreasing the degree of tyrosine protein phosphorylation reduces permeability of the blood-brain or other barrier, whereas increasing the degree of tyrosine protein phosphorylation increases permeability.

At page 6, please delete the paragraph appearing at lines 29-32, and substitute therefor the following paragraph:

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURES 1a, 1b and 1c show graphically that pervanadate decreases the transcellular electrical resistance (and by implication increases permeability) of strain I MDCK cells;

At page 9, please delete the paragraph appearing at lines 25-30, and the partial paragraph appearing at lines 32-34, and substitute therefor the following paragraph and partial paragraph:

FIGURE 19 shows a comparison of partial amino acid sequence data obtained for human p100 (SEQ ID NOs:1-5) with corresponding known sequence data from mouse p120 (SEQ ID NOs:1, 6-9). The letters given are based on the standard single letter amino acid code, apart from "X", which indicates any amino acid.

DETAILED DESCRIPTION OF THE INVENTION

In initial experiments, cells were treated with vanadate, a non-selective, but potent, inhibitor of tyrosine phosphatases. In MDCK cells, vanadate alone did not

At page 15, please delete the partial paragraph appearing at lines 15-34, and substitute therefor the following partial paragraph:

To address the issue of the identity of the catenin phosphorylated in response to PAO, peptide-directed antibodies were raised that specifically recognize α- or β-catenin. PAO-treated cells were lysed in SDS, followed by heating to dissociate protein complexes. Under these conditions only individual tyrosine phosphorylated proteins, not proteins associated with tyrosine phosphoproteins, are immunoprecipitated using anti-phosphotyrosine antibody. In such imunoprecipitates, β-catenin is rapidly increased in response to treatment of the cells with either PAO or, as expected, pervanadate (Fig. 6B). However, α-catenin was not detectable in the phosphotyrosine immunoprecipitates (Fig. 6C). Moreover, PAO- or even pervanadate-stimulated tyrosine phosphorylation of α-catenin could not be detected (Fig. 6E) in α-catenin immunoprecipitates (Fig. 6D). Thus, the tyrosine phosphorylation of β-catenin phosphorylation is increased in response to PAO, accounting for its immunoprecipitation by phosphotyrosine antibody. Furthermore, this increased phosphorylation appears to be

At page 17, please delete the partial paragraph that appears at lines 1-17, and substitute therefor the following partial paragraph:

an anti-ZO-1 antibody and tyrosine phosphorylation was examined by immunoblotting. PAO clearly stimulated the tyrosine phosphorylation of ZO-1 and to a lesser extent that of ZO-2 (Fig. 7). Pervanadate clearly resulted in the tyrosine phosphorylation of ZO-1, ZO-2 and, to a much lesser extent, that of a protein of 130 kDa (Fig. 7), possibly the same protein as that

identified by Balda, M.S., Gonzalez-Mariscal, L., Matter, K., Cereijido, M. and Anderson, J.M., 1993, Assembly of the tight junction: the role of diacylglycerol. *J. Cell Biol. 123:293-302.* These data illustrate that tight junction proteins as well as catenins are phosphorylated in response to PAO, raising the possibility that modulation of tight junction permeability could be achieved, either directly or indirectly, via changes in adherens junction adhesiveness and/or by direct modulation of tight junction permeability.

At page 33, please delete the paragraph appearing at lines 20-22 and substitute therefor the following paragraph:

EXAMPLES

The invention will now be illustrated by way of example only. In the following Examples the materials and methods discussed below are utilised.

At page 58, please delete the partial paragraph appearing at lines 3-8 and substitute therefor the following partial paragraph:

followed by heating at 100°C for 5 minutes. Proteins were precipitated by addition of four volumes of ethanol and incubation at -20°C for 16 hours. The precipitate was resolved by SDS-PAGE (6% acrylamide) and proteins were visualized by Coomassie Blue. Protein corresponding to p100 was excised from the gel and digested with LysC. Peptides were separated by HPLC and sequenced (SEQ ID NOs:1-10). Mouse p120 sequence was described by Reynolds et al., 1992. Clearly, human p100 is closed related to mouse p120 (see Fig. 19).

After page 71 and before the drawings, please insert the Abstract appended hereto as page 72.

After the drawings, please insert the sequence listing (pages 1-4) appended hereto.

In the Claims:

Please amend the claims as follows:

Please cancel claims 2-20, without prejudice or disclaimer to the subject material contained therein.

Please insert the following new claims 21-39:

21. (New) A method for increasing the permeability of a physiological barrier, comprising administering to a subject in need thereof an effective amount of an agent which promotes tyrosine protein phosphorylation.

22. (New) The method of claim 21, wherein the agent directly or indirectly activates tyrosine protein kinase.

23. (New) The method of claim 21, wherein the agent directly or indirectly inhibits tyrosine protein phosphatase.

24. (New) The method of either of claims 22 or 23, wherein the agent is a vanadium-containing salt.

25. (New) The method of claim 24, wherein the agent is a pervanadate.

26. (New) The method of either of claims 22 or 23, wherein the agent is phenylarsine oxide.

27. (New) A method for the treatment of brain oedema, comprising administering to a patient suffering therefrom an effective amount of an agent which promotes tyrosine protein dephosphorylation.

28. (New) The method of claim 27, wherein said brain oedema occurs as a result of stroke.

29. (New) The method of claim 27, wherein said brain oedema is associated with the occurrence of a brain tumor.

30. (New) A method for the treatment of peripheral oedema, comprising administering to a patient suffering therefrom an effective amount of an agent which promotes tyrosine protein dephosphorylation.

31. (New) The method of claim 30, wherein said peripheral oedema is high altitude pulmonary oedema.

32. (New) A method for blocking the entry into the brain of leukocytes that mediate an immune response, comprising administering to a patient in need thereof an effective amount of an agent which promotes tyrosine protein dephosphorylation.

33. (New) A method for the treatment of multiple sclerosis, comprising administering to a patient suffering therefrom an effective amount of an agent which promotes tyrosine protein dephosphorylation.

34. (New) A method for the prevention of cancer metastasis comprising administering to a patient in need thereof an effective amount of an agent which promotes tyrosine protein dephosphorylation.

35. (New) A method for increasing the transport of a membrane-impermeant compound across a physiological barrier, comprising the complexing of said compound with an agent which promotes tyrosine protein phosphorylation and administering the complex to a subject in need thereof, whereby the transport of said compound is increased.

36. (New) The method of claim 35, wherein said physiological barrier is an inter-endothelial cell tight junction.

37. (New) The method of claim 36, wherein said physiological barrier is the blood-brain barrier.

38. (New) The method of claim 36, wherein said physiological barrier is the vascularisation of a peripheral tumour.

39. (New) A composition comprising an agent which promotes tyrosine protein phosphorylation and a compound to be delivered across a physiological barrier.

Remarks

No new matter has been added by these amendments. Applicants have amended the specification to provide a title that is more descriptive of the subject invention (*i.e.*, the title as amended in the allowed parent application), to place the specification and claims into proper format for U.S. practice, to correct minor typographical errors in the specification (which were also corrected during prosecution of the parent case), to recite the sequence identification numbers next to the sequences in the text, and to direct the entry of the sequence listing into the appropriate location in the specification. Support for the foregoing amendments to the claims may be found throughout the specification as originally filed, and new claims 19-37 correspond substantially to claims 4 and 6-8 as originally filed in the application, and to claims 21, 22 and 27-39 which were added by preliminary amendment to the parent application prior to examination of that application on the merits. Hence, these amendments do not add new matter, and their entry and consideration are respectfully requested. Upon entry of the foregoing amendments, claims 1 and 21-39 are pending, with claims 1, 21, 27, 30, 32, 33, 34, 35 and 39 being the independent claims.

In accordance with 37 C.F.R. § 1.821, the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above application contain no new matter and are the same.

It is respectfully believed that this application is now in condition for examination. Early notice to this effect is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

In the Title:

The title of the application as filed is deleted and replaced by the following new title: -- Modulating the Permeability of a Physiological Barrier With an Agent that Modulates Tyrosine Phosphorylation --.

In the Specification:

In the specification at page 1, after the title, the following section is added:

-- CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of, and claims priority under 35 U.S.C. § 120 to, U.S. Appl. No. 08/648,182, filed December 23, 1997, which is a 371 of PCT/GB94/02543, filed November 18, 1994, the entire contents of which applications are incorporated herein by reference. --;

after the Cross Reference section and before the first line of the text (*i.e.*, before line 3), the following section and subsection headers are inserted:

-- BACKGROUND OF THE INVENTION

Field of the Invention --;

and at line 5, before the text of the second full paragraph on page 1, the following subsection header is inserted:

-- Related Art --.

In the specification at page 5, line 7 (*i.e.*, prior to the beginning of the first full paragraph of text on page 5), the following section header is inserted:

-- BRIEF SUMMARY OF THE INVENTION --.

In the specification at page 6, line 28 (*i.e.*, prior to the beginning of the paragraph appearing at lines 29-32), the following section header is inserted:

-- BRIEF DESCRIPTION OF THE DRAWINGS --.

In the specification at page 9, line 26, after "p100" the following is inserted: -- (SEQ ID NOs: 1-5) --;

at line 27, after "p120" the following is inserted -- (SEQ ID NOs: 1, 6-9) --; and

after line 30 (*i.e.*, prior to the paragraph beginning at line 32 and bridging pages 9 and 10), the following section header is inserted:

-- DETAILED DESCRIPTION OF THE INVENTION --.

In the specification at page 15, line 21, please delete "associates" and substitute therefor -- associated --.

In the specification at page 17, line 14, after "achieved" please insert -- , -- (a comma); and after "either" please delete the comma and insert therefor -- directly or --.

In the specification at page 33, line 19 (*i.e.*, before the paragraph appearing at lines 20-22), the following subsection header is inserted: -- EXAMPLES --.

In the specification at page 58, line 7, after "sequenced" please insert -- (SEQ ID NOs: 1-9); and at line 8, after "p120" please insert -- (see Fig. 19) --.

After page 71 and before the drawings, the Abstract appended hereto as page 72 is inserted.

After the drawings, the sequence listing appended hereto (pages 1-4) is inserted.

In the Claims:

Claims 2-20 are sought to be canceled without prejudice or disclaimer.

New claims 21-39 are sought to be entered.

Modulating the Permeability of a Physiological Barrier With an Agent that Modulates Tyrosine Phosphorylation

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of, and claims priority under 35 U.S.C. § 120 to, U.S. Appl. No. 08/648,182, filed December 23, 1997, which is a 371 of PCT/GB94/02543, filed November 18, 1994, the entire contents of which applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to the control of permeability of the blood-brain barrier and other physiological barriers.

Related Art

The blood-brain barrier serves to separate the molecular, ionic and cellular environment of the blood from that of the brain. To a major degree, this separation is achieved by inter-endothelial tight junctions of high electrical resistance which greatly diminish paracellular flux. It is clear that the permeability of the tight junctions of the blood-brain barrier is not immutable. Rather, permeability appears to undergo dynamic regulation, especially by second messenger pathways.

Acquiring the ability to manipulate the permeability of the tight junctions of the blood-brain barrier is important for a number of reasons, among which are the following:

(i) To decrease brain oedema following stroke by closing the tight junctions of the blood-brain barrier;

(ii) To deliver blood-borne, membrane-impermeant drugs to the brain by reversibly opening the tight junctions of the blood-brain barrier; and

(iii) To block the entry into the brain of both leukocytes that mediate an immune response, such as occurs in multiple sclerosis, and metastatic cancer cells that may form tumours. (It is believed that during cell trafficking across the endothelium, the migrating cell passes through the tight junction